METABOLIC STABILITY STUDY OF ANTITUMOR PEPTIDE CIGB-552 IN BALB/C MICE SERUM

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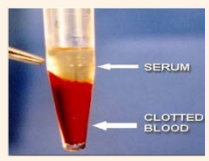
Abstract

The most important limitations of therapeutics peptides are their short half-life in vivo due to degradation by peptidases and their high speed clearance through the liver and kidneys. Despite these disadvantages, therapeutics peptides have become in an interesting approach to confront health problems like hormones or metabolic disorders, infectious diseases and cancer. That´s why it is very important to know where are peptide vulnerability within the sequence, previous to their development as a therapeutic candidate. The metabolic stability studies in vitro of these molecules can provide us of useful information about their latest behavior in vivo[1]. In this work we assayed the stability of antitumor peptide CIGB-552 in Balb/c mice serum, at 37 ºC. Peptide degradation profile in serum, up to two hours, was obtained. The half-life time in vitro was calculated by triplicate as previously reported[2]. Mean value was 22.23 ± 0.68 minutes. Typical serine-proteases degradation pattern was suggested for this peptide. Based on mass spectrometry characterization from resulting main metabolites, enzymes found to be responsible for CIGB-552 degradation, were essentially: Tripsin, Quimotripsin and Pancreatic elastase. It was confirm by RP-HPLC for Tripsin and Quimotripsin. In the long term stability assay (up to 24 hours at 37 ºC), it was found that metabolite containing D-amino acids substitutions in the sequence (highlighted in red), was detected until 8 hours at 37 ºC. This finding support the assumption of D-amino acids protects the sequence against enzymatic degradation[3].

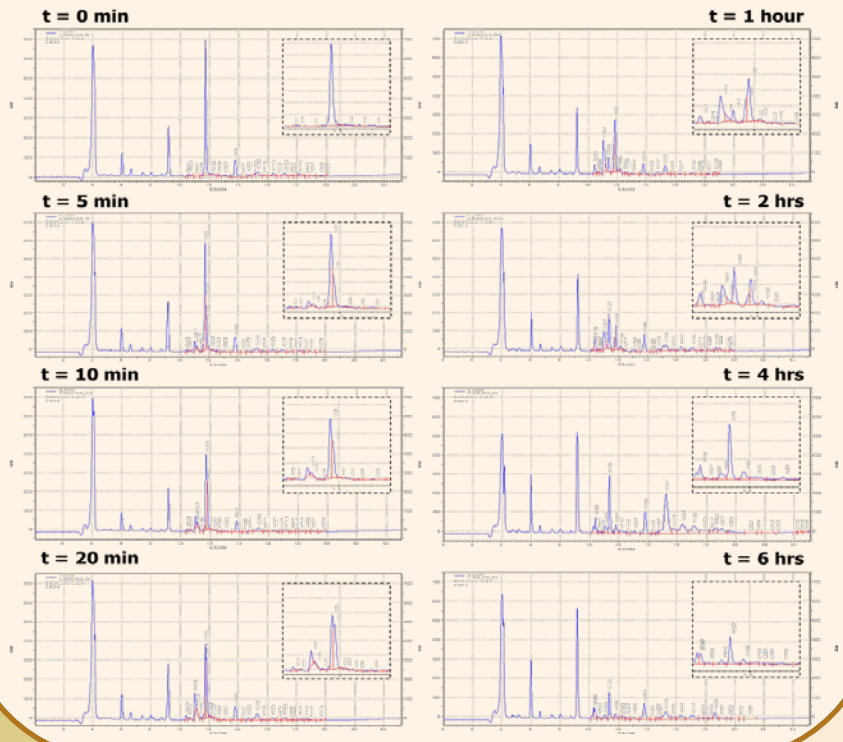
Experimental procedure

Sample Processing

1. Protein precipitation: TFA (10% final conc.)
2. Vortex 1 min
3. Centrifugation 14 000 rpm x 5 min
4. Supernatant recovery

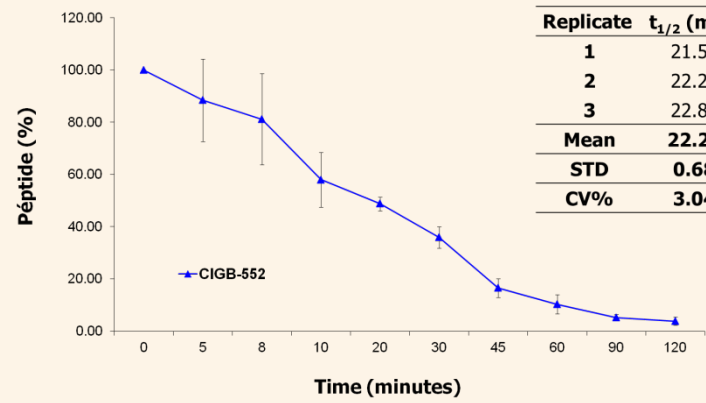


Degradation kinetics



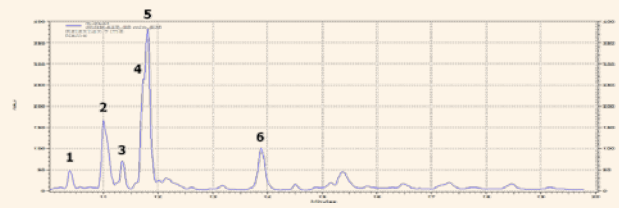
Results

Degradation profile and $t_{1/2}$ calculated



Replicate	$t_{1/2}$ (min)
1	21.53
2	22.29
3	22.88
Mean	22.23
STD	0.68
CV%	3.04

Main fractions characterized/identified



Fraction	MM (g/mol)	Identification by Mass Spectrometry
1	1820.19	IKPTFRRLKWKYKG
	2226.30	HARIKPTFRRLKWKYKG
2	1635.04	IKPTFRRLKWKY
	1750.17	HARIKPTFRRLKW
	2041.08	HARIKPTFRRLKWKY
3	1343.94	IKPTFRRLKW
4	2686.42	HARIKPTFRRLKWKYKGKFW **(CIGB-552)**
5	2280.17	IKPTFRRLKWKYKGKFW
6	-	Non related peptide (from serum)

Degradation pattern suggested

Conclusions

1. CIGB-552 half life in mice serum was 22.23 ± 0.68 minutes.
2. Typical serine-proteases degradation pattern was suggested for this peptide in this biological fluid.
3. D-amino acids within the sequence, contribute to metabolite (3) persistence in serum, relative to the original peptide and other intermediaries

References

1. Journal of Proteome Research (7): 5112-5118 (2008).
2. Journal of Pharmacology and Experimental Therapeutics 319 (1): 308-316 (2006).
3. Pharmaceutical Research 10 (9): 1268-1273 (1993).

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